29. PRION - NEW ANSWER OR THE OLD RIDDLE

Josip Talapko, GS OSRH, Osijek, Ankica Čižmek, HVU, UHKoV "Fran Krsto Frankopan", Ilica 256 b, Zagreb

ABSTRACT

Life, like the eternal and unsolved riddle, and the Science, which tries to find the answers, are both the inspires to each other. The interrelations between live and live, and life and death, move us towards the question about the beginning (or maybe the end) of life.

Where is the place of PRION in all these philosophical thinking? It now appears that an infectious agent named a prion may stand out as a remarkable exception to the rule that every organism carries nucleic acid defining its own identity. The prion is known as capable of initiating the production of new prions, at least in certain mammalian cells.

Prion diseases are the group of neurological diseases known in humans as: curu, sCJD, fCJD, ICJD, nvCJD, GSS, FI, and in animals as: TME, CWD, FSE, BSE and SCRAPIE.

Most of these diseases became by ingestion of by products of sheep that have the disease to scrape off much of their wool. Changes brought on by the disease are confined to the central nervous system. A consistent indicator is abnormal proliferation of the astrocytes, a class of supporting cells in the brain. In neurons there is a depletion of dendritic spines, which have a role in the transmission of nerve impulses. In some of the disorders numerous vacuoles give the brain tissue a spongy appearance.

Prions contain protein and reproduce in the living cell, yet no DNA or RNA has been found in them. What is the nature of their genome?

Could these facts can be the possibility of creation of new agents for terrorists, and could the mechanisms of the most hidden secrets of the life be used to create evil?

Are prions potential BUG of the 21. century, and are we , humans, capable of making antidote for them?

INTRODUCTION

WHAT ARE PRIONS?

The prion protein (PrP^c) is a glycolipid-anchored, cell surface protein of unknown function, a posttranslationally modified isoform of which has been implicated in the pathogenesis of spongioform encephalopathies in man and animals.

The term "prion" was coined by Prusiner to indicate an infectious agent with protein like properties.. The unusual properties of the pathogen were demonstrated in early experiments in which conditions that degrade nucleic acids, such as exposure to ionizing and ultraviolet radiation, did not reduce the infectivity of scrapie fractions, on the other hand, treatments that degrade protein, such as prolonged exposure to proteases, correlated with a reduction in infectivity. A protein with relative resistance to protease digestion was found to be consistently present in the brains of animals and humans with TSE. Surprisingly, this protein was found to be one that is normally encoded by a chromosomal gene of the host.

Prion diseases are a group of fatal neurodegenerative disorders that can occur in hereditary, sporadic, and infectious forms.

These illnesses occur in humans and a variety of other animals. Prions are infectious proteins. The normal cellular form of the prion protein (PrP) designated PrP^c, contains three α- helices and has little β- sheet; in contrast, the protein of the prions, denoted the scrapie form of PrP (PrP^{Sc}), is rich in β- sheet structure. The accumulation of PrP^{Sc} in the central nervous system precedes neurological dysfunction accompanied by neuronal vacuolation and

astrocytic gliosis. PrP is highly conserved, host- encoded sialoglycoprotein that may play a role in normal synaptic function and circadian rhythms. This host protein (PrP^c) is present in the brain and other tissues and is sensitive to proteinase K (PK) digestion.

The spongioform encephalopaties are a group of transmissible, neurodegenerative disorders including kuru, Creutzfeldt-Jacob disease, and Gerstmann-Sträussler syndrome in man, and scrapie and bovine spongioform encephalopathy in animals.

The infectious agent (prion) responsible for these diseases is composed primarily, if not exclusively, of the protein PrP^{Sc}, which is a posttranslationally altered isoform of the normal cellular protein PrP^c (Prusiner, 1991.). Although the structural features that distinguish the two isoforms have not yet been identified, they differ in several biochemical properties, including resistance to protease digestion and solubility in detergents. Recent evidence also suggests cell biological differences. PrP^{Sc} is found primarily inside infected cells where it appears to accumulate, while PrP^c is a surface protein, which is degraded with a half-life of several hours.

The physiological function of PrP^c has remained elusive. It is abundantly expressed in the central nervous system and in several peripheral tissues beginning early in embryonic development, and it has been suggested that the protein plays a role in neural differentiation, lymphocyte proliferation or cell adhesion.

How does PrP^c convert to PrP^{sc}?

Potential mechanisms that initiate conversion of PrP^c to PrP^{Sc} include a germ line mutation of the human prion protein gene (PRNP), a somatic mutation within a particular neuron, and spontaneuos conversion of PrP^c to an aberrant conformation, that is not refolded appropriately to its native structure. Regardless of the initiating event, once an "infectious unit" has been generated, PrP^{Sc} appears to act as a conformational template by which PrP^c is converted to a new molecule of PrP^{Sc} through protein-protein interaction of PrP^{Sc} and PrP^c (Fig.1A and 1B). This concept is supported by several studies which show that mice with the normal PrP gene deleted (PrP knockout mice) do not develope prion disease after inoculation with scrapie. Further more, transgenic (Tg) mice that express a chimeric PrP gene made of human (Hu) and mouse (M) segments, designated Tg(MHu2M), develop protease-resistant chimeric mouse-human PrP^{Sc} (i.e., $MHu2MPrP^{Sc}$) in their brains, when inoculated with brain extracts from humans with prion disease. These findings clearly ilustrate that prions do not self-replicate but instead convert nonpathogenic PrP^c to pathpgenic PrP^{Sc}.

Conversion of the cellular prion protein (PrP^c) to an abnormally confirmed, aggregated, protease-resistant isoform (PrP^{Sc}) is a cardinal feature of a prion diseases. In humans, PrP^c comprises 209 amino acids, a disulfide bridge between residues 179 and 204, a glycosylphosphatidylinositol anchor, and two sites of non-obligatory N-linked glycosylation.

Bovine spongioform encephalopathy (BSE) is a transmissible spongioform encephalopathy (TSE) or prion disease of cattle first recognized in 1986. in the United Kingdom, where it produced a common source epidemic that peaked in January 1993 and has subsided markedly since that time.

The epidemic began simultaneously at many geographic locations and was traced to contamination of meat and bone meal (MBM), a dietary supplement prepared from rendering of slaughterhouse offal. It appears that the epidemic was initiated by presence of the agent of scrapie (a long-standing TSE of sheep) that was first transmitted to cattle, beginning in the early 1980s, when most rendering plants abandoned the use of organic solvents in the preparation of MBM. The epidemic was probably accelerated by the recycling of infected bovine tissues prior to the recognition of BSE. The NMR structure of the recombinant bPr (23-230) (Fig. 3) contains a globular domain that extends approximately from residue 122 to

residue 227, where the residues 128-131 form the β-strand 1,144-154 the α-helix 1 (which has 3 10-type structure from sresidue 153 onward), 161-164 the β-strand 2, 173-194 the α-helix 2, and 200-226 the α-helix 3. There is currently no effective therapy for human prion diseases, although several chemotherapeutic agents have been tested in animal models (Pocchiari et al., 1991.; Ingrosso et al., 1995.). Polyanionic glycans such as pentosan sulfate (PS) and dextran sulfate (DS) have been among the most intensively studied. These agents were initially tested because they were known to be active against conventional DNA and RNA viruses, but it was found that they were also infective in vivo against infection by scrapie prions, prolonging the incubation time, and in some cases, completely preventing the developement of simptoms when administered prophylactically to mice and hamsters. Also, the addition of copper facilitated restoration of both infectivity and protease resistance of PrP in a subset of samples that did not renature by the simple dilution of GdnHCl. These data demonstarte that lose of scrapie infectivity can be a reversible process and that copper can enhance this restoration of proteinase K resistance and infectivity.

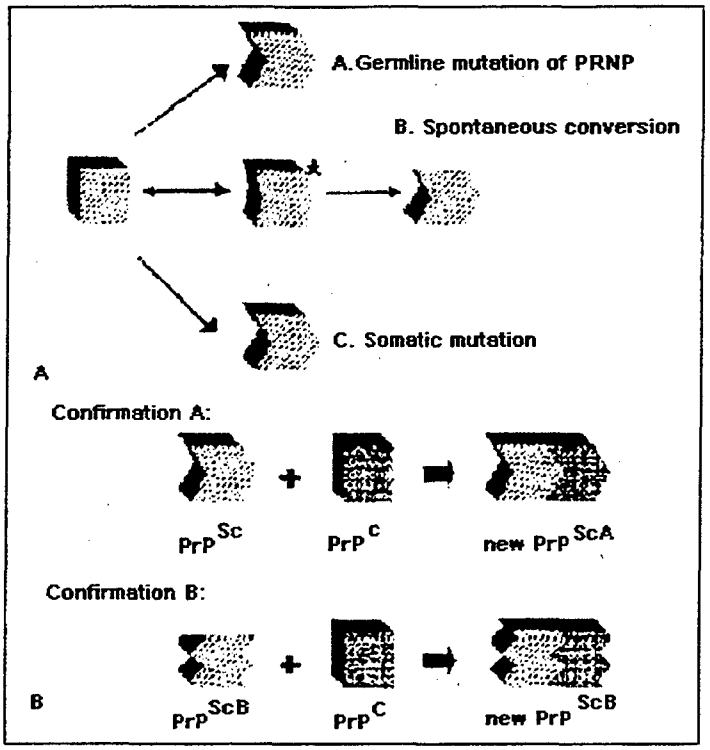
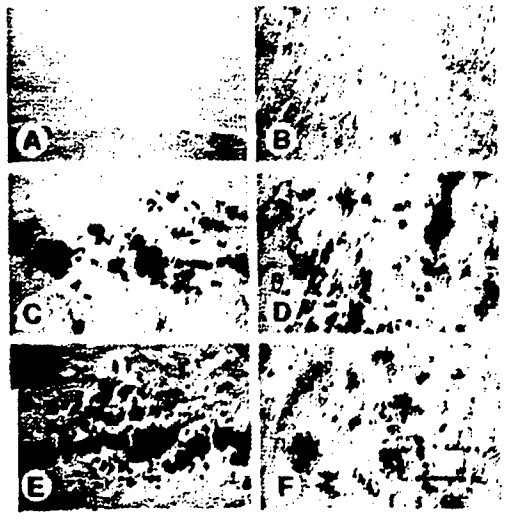
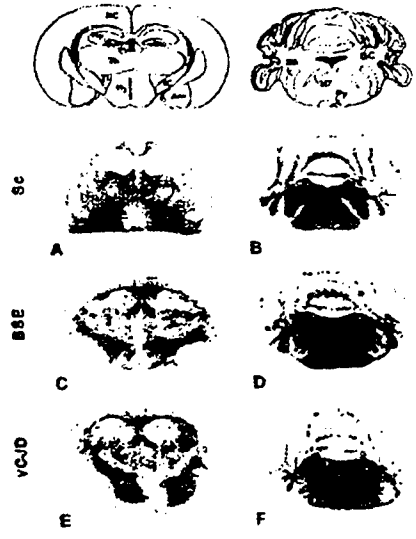


Figure 1. (A) Potential mechanisms by which conversion to PrP^{Sc} is initiated. In humans three potential mechanisms are postulated to give rise to PrP^{Sc}. A, Germline mutation: The genetic varieties of disease are most easily explained by a mutation of the PRNP gene that acts to destabilize PrP, which in turn leads to the generation of PrP^{C}. B, Somatic mutation: A mutation may occur withnin a single cell or group of cells in the brain to induce the disease-causing conformation of PrP. C, PrP may naturally adopt an intermediate unstable form (designated by the star) that can be converted relatively easily to the native state or the pathogenic state. This may depend on factors within the cell that help to stabilize or destabilize PrP. (B) PrP^{Sc} acts as a conformational template to generate new PrP^{Sc} with a similar conformation. PrP^{Sc} appears to acquire several pathogenic conformational subtypes, which may help explain the diverse phenotypes of prion disease. Once PrP^{Sc} is generated by any of the mechanisms described in (A) or through an as yet undetermined mechanism, it induces the conversion of nonpathogenic PrP^{C} to PrP^{Sc} by interaction of the two protein conformations. In this example, PrP^{Sc} is shown in two potential conformations. PrP^{Sc} with conformation A generates new PrP^{Sc} with conformation A, and PrP^{Sc} with conformation B generates new PrP^{Sc} with conformation B. This property helps to explain how prion strains with characteristic phenotypic properties are transferred and maintained.

Figure 2. Pathohistolic preparations at prion deseases





In these mice, thc pattern of neuropathology differed markedly; inocula derived from sheep scrapie resulted in a mild degree of vacuuolation in virtuallyall brain regions and no amyloid plaquse (A)

One neuropathological difference was the presence of PrP-immunopositive amyloid plaques, primarily in the subcallosal region in the present study and their absence in the first passage (C)

Specifically, the nonamyloid PrP deposits with the latter were of the coarse type (D and F), whereas they were of the finely granular, "siynaptic" type with scrapie (B)

Prp - immunopositive amyloid plaques vere also found in the subcallosal region (E)

The vacuolation profiles with each prion inoculation correlated well with the PrPsc distribution profiles. Common to all three inocula was uniformly moderate degree of vacuolation in the brainsterm tegmentum and periaqueductal gray that coresponded to intense immunostaining for PrPsc in the same regions (compare B,D and F).A moderate degree of vacuolation found in the habenula in mice inoculated withBSE and nv CJD also correlated with intense PrPsc immunostaining (C and E) , whereas there was very modest vacuolation in the habenula of mice inoculated with scrapie prions, a result consistent with the lower intensity ofPrPsc staining observed (A).

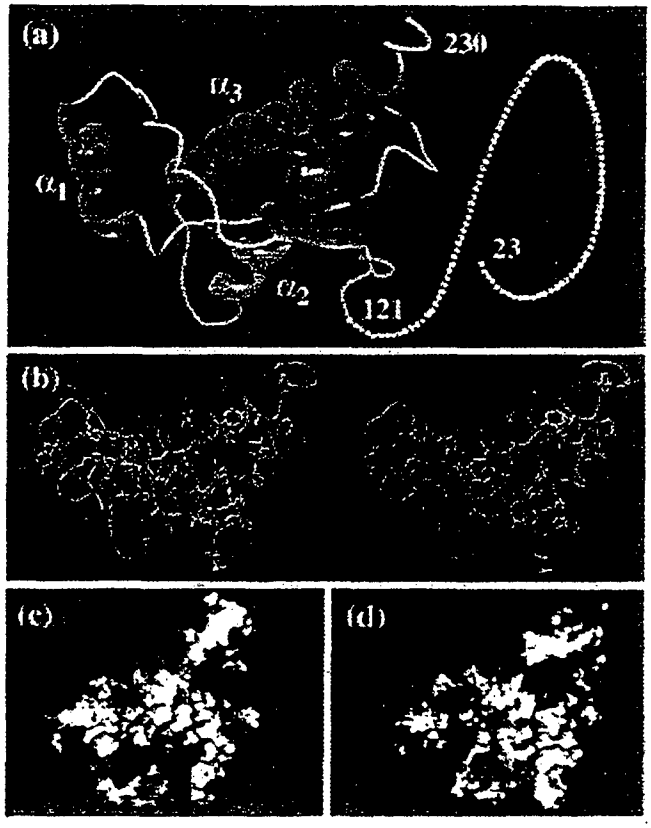


Fig. 3. (a) Cartoon of the three-dimensional structure of the intact bPrP(23-230). Helices are green, ß-strands are cyan, segments with nonregular secondary structure within the C-terminal domain are yellow, and the flexibly disordered "tail" of residues 23-121 is represented by 108 yellow dots, each of which represents a residue of the tail (the numeration for hPrP is used, and the insertions and deletions are placed according to the alignment in ref.23).(b) Stereo-view of an all-heavy atom presentation of the globular domain in bPrP(23-230), with residues 121-230, in the same orientation as in a. The backbone is shown as a green spline function through the C° positions, hydrophobic side chains are yellow, and polar and charged side chains are violet.(c and d) Surface views of the globular domains of the bPrP and hPrP, respectively. The orientation of the molecule is slightly changed relative to a, so that the residue 186 is approximately in the center. The electrostatic surface potential is indicated in red (negative charge), white (neutral), and blue(positive charge).
The figures were prepared with the program MOMOL (42).

Table 1. The Clinical Phenotypes of prion Diseases

Disease	Primary Features	Host	Duration	Mechanism of Pathogenesis
Kuru	Ataxia dementia	Human	3 months to 1 year	Infection through ritualistic cannibalism
iCJD Iatrogenic Creutzfeldt-Jacob disease	Ataxia, dementia	Human		Infection from prion-contaminated HGH, dura mater grafts, and so forth
sCJD Sporadic Creutzfeldt-Jacob disease	Demantia, ataxia, myocolonus	Human	< 1 y	Somatic mutation or spontaneuos conversion of PrP^{c} ionto PrP^{Sc}
fCJD Familial Creutzfeldt-Jacob disease	Demantia, ataxia, myocolonus	Human	1-5 y	Germline mutations in PrP gene
GSS Gerstmmann- Sträussler-Scheinker disease	Ataxia, late dementia	Human	2-6 y	Germline mutations in PrP gene
FI Fatal familial insomnia	Insomnia, dysautonomia, ataxia, dementia	Human	~ 1 y	Germline mutations in PrP gene (D178N and M129)
vCJD Variant Creutzfeldt-Jacob disease	Behavioral changes, late dementia	Human	~ 1.5 y	Infection from bovine prions=?
Scrapie	Behavioral changes	Sheep		Infection in geneticaly susceptible sheep
BSE Bovine spongioform encephalopathy	Behavioral changes	Cattle		Infection with prion-contaminated MBM
TME Transmissible mink encephalopathy	Behavioral changes	Mink		Infections with prions from sheep or cattle
CWD Chronic wasting disease	Behavioral changes	Mule deer, elk		Unknown
FSE Feline spongioform encephalopathy	Behavioral changes	Cats		Infection with prion-contaminated MBM
EUE Exotic ungulate encephalopathy	Behavioral changes	Greater kudu, nyala, oryx		Infection with prion-contaminated MBM

Table 1. The Prion Diseases - (Prusiner, 1998.)

Table 2. Reported cases of bovine spongiform encephalopathy in the United Kingdom and other countries (as of December 2000)

Country	Native cases	Imported cases	Total cases
United Kingdom	180,376[b]	0	180,376
Republic of Ireland	487	12	499
Portugal	446	6	452
Switzerland[c]	363	0	363
France[c]	150	1	151
Belgium	18	0	18
Netherlands	6	0	6
Liechtenstein	2	0	2
Demnark	1	1	2
Luxembourg	1	0	1
Germany	3	6	9
Oman	0	2	2
Italy	0	2	2
Spain[d]	0	2	2
Canada	0	1	1
Falklands (IJK)	0	1	1
Amres (Portugal)[e]	0	1	1

[a]Data from Organization of Internatioinal Epizootics(Paris) and Ministry of Agriculture, Fisheries, and Food (UK).
[b]Includes 1,287 cases in offshore British islands.
[c]Includes cases detected by active surveillance with immunotogic methods.
[d]Origin and dates of imported cases are under investigation.
[e]Case imputed from Germany.

Table 3 Summary of iatrogenic cases of Creutzeldt-Jakob disease from all causes (July 2000)

Mode of infection	No. of patients	Agenl entry into brain	Median incubation period (renge)*	Clinical signs on presentation
Corneal transplant[#]	3	Optic nerve	16, 18, 320 mo	Dementia/ cerebellar
Stereotactic EEG	2	Intracerebral	16, 20 mo	Dementia/ cerebellar
Nearosurgery	5	Inlracerebral	17 mo (12-28)	Visual/dementia/ cerebellar
Dura mater grail	114	Cerebral surface[$]	6 y (1.5-18)	Cerebeller(visual /dementia)
Growth hormone	139	Hematogenous	12 y (5-30)	Cerebellar
Gonadotropin	4	Hematogenous	13 y (12-16)	Cerebellar

*Calculated from the midpoint of treatment to the onset of disease.
[#]One defnite, one probable, and one possible case.

[5]In two cases, dura was used to embolize vessels of non-CNS tissues, rather than as intracranial grafts.

CONCLUSIONS

BSE were not noted down as new diseases. In fact, a man started playing with nature feeding plant-eating animals with proteins of animal origin and making in such a way causes of infection as well as making ways for expansion of the infection.

Is it possible to conclude that circumstances of rising of prion diseases (genetic, biochemical, infections, epidemiological, economic, and other) can be abused in making a "super BUG".

Some of conditions and criterions for BUG,s employment are:

- -Possibility of provocation action on as larger as possible territory, massiveness in fact;
- -Selectivity related on time, effects (medical, economical, safety, defensive) and population categories;
- -Usage of self-transferred agents either directly or indirectly (through vector-domestic animal or game, mischief-doer;
- -Masked of symptoms until wanted level of infection has been reached;
- -Non-existing of specific protection;
- -Hiding of the doer.

Modern science, especially biology (genetics, medicine) gives us deeper and deeper answers on life functions, relationships of kinds but also blame some new possibilities. Biotechnology of today promises rising of living quality, and imitating evolution, tries to make a human sure that is a self-sufficient sort.

However, non-perfection of global relations in society makes balance between those that have economical, political and military advantage and those that would like to have one much weaker.

This is a cruel battle on the old and new "battle-fields". Terrorism, in fact bioterrorism as his the most perfect form, may change the face of the world in the most literal sense of the word. If there is a political decision of any interested group, technical circumstances for doing this (bioterrorism) do not make any problem.

Prion diseases (TSE, BSE) as it is known threw down on their knees the most developed countries of Europe, which in economical, medical, justical and safety sense tries to find out the right answers.

If we, nevertheless, conclude that these events are the result of lack of judgement and coincidence, these prognosis are even worst, because if these tragedies are going on, even unintentionally, what we can expect when international terrorism get involved.

Certainly, now when the principle of men genetic function are known, and when it is easy act on some diseases it is also easy act on "SUPER BAG".

That because Prion and its diseases will be going on for some time and men have to approach on it like any other armies.

MAN has to be in the middle of the close attention and it is necessary to arm by knowledge and will for humanization and prosperity of person in society.

Only then we could prevent and isolate common safety system, where separate excesses (native and social) could not become a global danger.

KEY WORDS

Prion, TSE, mutation

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